Environmental Research 109 (2009) 239–244

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Electrophilic and redox properties of diesel exhaust particles ☆

Masaru Shinyashiki [a,e], Arantza Eiguren-Fernandez [a,e], Debra A. Schmitz [a,e], Emma Di Stefano [a,e], Ning Li [c], William P. Linak [d], Seung-Hyun Cho [d,f], John R. Froines [a,b,e], Arthur K. Cho [b,e,*]

[a] Center for Occupational and Environmental Health, School of Public Health, University of California, Los Angeles, Los Angeles, CA 90095, USA
[b] Department of Environmental Health Sciences, School of Public Health, University of California, Los Angeles, Box 951772, 21-297 CHS, Los Angeles, CA 90095-1772, USA
[c] Division of NanoMedicine, Department of Medicine, David Geffen School of Medicine at , Los Angeles, CA 90095, USA
[d] The Air Pollution Technology Branch, National Risk Management Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA
[e] The Southern California Particle Center, School of Public Health, University of California, Los Angeles, Los Angeles, CA 90095, USA
[f] Oak Ridge Institute for Science and Education (ORISE), USA

ARTICLE INFO

Article history:
Received 19 August 2008
Received in revised form
24 October 2008
Accepted 22 December 2008
Available online 6 February 2009

Keywords:
Diesel exhaust particles
Electrophiles
Redox activity
Humic-like substances
Dithiothreitol
GAPDH

ABSTRACT

The adverse health effects of air pollutants have been associated with their redox and electrophilic properties. Although the specific chemical species involved in these effects are not known, the characterization of their general physical and chemical properties is important to our understanding of the mechanisms by which they cause health problems. This manuscript describes results of a study examining the partition properties of these activities in aqueous and organic media. The water and dichloromethane (DCM) solubility of redox active and electrophilic constituents of seven diesel exhaust particle (DEP) samples were determined with assays developed earlier in this laboratory. The constituents exhibiting redox activity, which included both metals and nonmetal species, were associated with the particles in the aqueous suspensions. Portions of the redox active compounds were also DCM-soluble. In contrast, the electrophilic constituents included both water-soluble and DCM-soluble species. The role of quinones or quinone-like compounds in redox and electrophilic activities of the DCM-soluble constituents was assessed by reductive acetylation, a procedure that inactivates quinones. The results from this experiment indicated that most of the activities in the organic extract were associated with quinone-like substances. The partition properties of the reactive species are important in exposure assessment since the toxicokinetics of particles and solutes are quite distinct.

1. Introduction

The adverse health effects of air pollutants have been attributed to the ability of these pollutants to induce cellular oxidative stress through the generation of reactive oxygen species (Dagher et al., 2006; Donaldson et al., 2003; Imrich et al., 2007; Li et al., 2003a, b; Rahman et al., 2006). However, the chemical basis for the oxidative stress induction is not clear. This laboratory has been studying the chemical properties of airborne particles and vapors to characterize those components that contribute to these adverse effects. In the course of this work, analytical procedures to assess the redox capacity of particulate matter and diesel exhaust particle (DEP) were developed (Cho et al., 2005). Subsequently, an assay for the electrophile content of air samples was developed, since electrophiles can also contribute to the induction of oxidative stress by altering the ratio of concentrations of oxidized to reduced antioxidants such as glutathione (Shinyashiki et al., 2008). More recently, an assay for the capacity of a given sample to catalyze the Fenton reaction, which generates hydroxyl radical, has been developed (Di Stefano et al., 2009). The original studies were based on the hypothesis that quinones were key organic compounds likely to cause oxidative stress by either redox cycling or conjugation with thiols. Accordingly, we first demonstrated quinone presence in DEP by direct analysis (Cho et al., 2004), then demonstrated that ambient particles and vapors could participate in the same chemical reactions as certain quinones. For example, the redox active quinone, 9,10-phenanthroquinone (PQ), was shown to be capable of catalyzing electron transfer between the

☆ *Funding source*: The research described in this article has been funded in part by the United States Environmental Protection Agency through Grant no. CR-82805901 to UCLA. While the EPA co-authors collected the DEP samples under an approved quality assurance project plan, the subsequent analyses have not been subjected to the Agency's required peer and policy review. The contents of this article should not be construed to represent Agency policy and no official endorsement should be inferred. The work has also been supported by The Asthma and Outdoor Air Quality Consortium of the South Coast Air Quality Management District (Sponsor award no.: 040621), and by the Southern California Environmental Health Science Consortium (SCEHSC) 5P30 ES07048 (funded by NIEHS).
* Corresponding author at: Department of Environmental Health Sciences, School of Public Health, University of California, Los Angeles, Box 951772, 21-297 CHS, Los Angeles, CA 90095-1772, USA. Fax: +1 310 206 9903.
E-mail address: Acho@mednet.ucla.edu (A.K. Cho).

0013-9351/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2008.12.008

dithiol, dithiothreitol (DTT) and oxygen, generating superoxide, and thence hydrogen peroxide (Kumagai et al., 2002). Analogously, airborne and DEP, as well as ambient vapors, carry out similar reactions (Cho et al., 2005), indicating that these samples contain chemical species which, like PQ, will catalyze electron transfer. In addition, the electrophilic quinone, 1,4-benzoquinone, was shown to react with cellular thiols such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and irreversibly inactivate it by covalent bond formation (Rodriguez et al., 2005). This effect led to the development of an assay to demonstrate the presence of electrophiles in ambient air samples by utilizing the ability of electrophiles to inactivate this enzyme through alkylation (Shinyashiki et al., 2008).

This manuscript describes results of a study in which the procedures above were used to characterize the physical and chemical properties of seven DEP samples collected by the Air Pollution Technology Branch of the US Environmental Protection Agency. Initially, the polycyclic aromatic hydrocarbon (PAH) and quinone content of the organic extracts of the particles were determined. Then, the physical properties of the redox species in the samples were examined by comparing the distribution of activities in dichloromethane (DCM) extracts and aqueous suspensions of the particles. Finally, the chemical nature of the reactive species was assessed by subjecting the organic extracts to reductive acetylation, a procedure that converts quinones to their relatively unreactive hydroquinone diacetates.

2. Materials and methods

Chicken muscle GAPDH, NAD^+, EDTA, glyceraldehyde-3-phosphate (G-3-P), dithiothreitol (DTT), 5-5′-dithiobis(2-nitrobenzoic acid) (DTNB), 2,3- and 2,5-dihydroxybenzoic acids (DHBAs), sodium salicylate, ascorbic acid, diethylenetriaminepentaacetic acid (DTPA), citric acid trisodium salt dihydrate and monohydrate, cupric sulfate pentahydrate, ferrous ammonium sulfate and the quinone standards were purchased from Sigma-Aldrich Co. (St. Louis, MO). Other reagents were of reagent or the highest grade available and obtained from Fisher Scientific (Pittsburgh, PA). Polycyclic aromatic hydrocarbon standards were purchased from Cerilliant Corporation (Round Rock, TX). Mouse macrophage cell line (RAW 264.7) cells were obtained from American Type Cell Culture (Manassas, VA). DMEM, fetal bovine serum and penicillin/streptomycin were purchased from Invitrogen (Carlsbad, CA). Monoclonal anti-heme oxygenase-1 (HO-1) antibody was from Stressgen (Ann Arbor, MI). Chelex 100 was from Bio Rad (Hercules, CA).

2.1. DEP samples

The seven DEP samples examined were generated at the US EPA's National Risk Management Research Laboratory (RTP, NC) as part of a project to characterize the chemical, physical and toxicological properties of combustion generated particles. EPA collected the samples but the subsequent analyses were performed at UCLA. The samples were collected over a period from June 2004 to October 2005 during seven separate animal inhalation exposure experiments lasting between 5 and 39 days. One sample, DEP4, was collected between January 24 and 28, 2005, and exhibited higher levels of organic species such as the PAHs and quinones. DEP was generated using a 30 kW (40 hp) 4-cylinder indirect injection Deutz diesel engine (Model BF4M1008) under load of a 22.3 kW (30 hp) Saylor-Beall air compressor (Model 707). Readily available road taxed diesel fuel (analysis not shown) was supplied via a re-circulating loop from a 208 L (55 gal) drum. Engine lubrication oil (Shell Rotella, 15W-40) was changed before each of the seven exposure experiments. The engine (~1725 rpm) and the compressor were operated at steady-state to produce 0.8 m^3/min (30 ft^3/min) of compressed air at 400 kPa (60 psig). This translates to approximately 20% of the engine's full-load rating. Fuel consumption was 7.6–11.4 L/h (2–3 gal/h). The engine and the compressor were operated outdoors under conditions of varying temperature, humidity and precipitation including both winter and summer months. From the engine, a small portion of the exhaust was routed to a dilution system and directed to exposure chambers. The remaining exhaust was diluted with ambient air (~3:1) to near ambient temperatures (~35 °C, ~95 °F) and directed to a small 4.2 m^3/min (150 ft^3/min) rated Dustex baghouse (Model T6-35-9) containing nine Nomex felt bags. The bags were periodically reversed pulsed using compressed air to remove the accumulated DEP which were collected from the hopper at the end of each day and stored refrigerated (4 °C, 40 °F) in glass sample jars. The DEP mix sample was prepared by mixing 100 g samples from each DEP3, DEP5, and DEP7 in a 19 L (5 gal) plastic bucket using a bucket tumbler operated at 25 rpm for 45 min.

2.2. Sample preparation

2.2.1. Aqueous suspensions and filtrates

The DEP samples (0.7–6.5 mg) were weighed in glass vials and deionized water (DIW) was added to achieve concentrations of 0.08–0.5 mg/mL. The suspensions were sonicated for 15 min in a water bath. The bath water was replaced to prevent heating of the samples and the suspensions were sonicated for an additional 15 min. Filtrates were made by filtering the aqueous suspensions through a 4 or 25 mm 0.45 μm nylon syringe filter (Nalgene, NY).

2.2.2. Organic extracts

The DEP samples were extracted with Optima grade dichloromethane by sonication for 30 min. The resulting suspensions were then filtered through a 0.45 μm filter (Millipore, Billerica, MA) and aliquots were separated for the different chemical and toxicological assays.

To prepare organic extract suspensions for the chemical and biological assays conducted in aqueous media, the DCM extract was evaporated and reconstituted in dimethyl sulfoxide (DMSO).

2.2.3. Reductive acetylation of DEP extracts

The organic extracts of the DEP samples were subjected to the reductive acetylation procedure used in the quinone assay (Cho et al., 2004). Thus, DCM extract was concentrated to approximately 50 μL by evaporation and approximately 200 mg of powdered zinc together with 200 μL of acetic anhydride in 400 μL tetrahydrofuran was added. The mixtures were then heated for 15 min at 80 °C. The samples were cooled and an additional 200 mg of zinc was added and the mixture was heated for an additional 15 min. The reaction was quenched by the addition of 500 μL of water and 3.0 mL of pentane. The pentane layer was concentrated by evaporation and the residue reconstituted in DMSO. The final concentration of the extract used in the assays was 100 μg/μL. When necessary, the concentrated samples were diluted to insure that the results remained within the linear portion of concentration effect curves.

2.3. Chemical assays

The content of PAHs (Eiguren-Fernandez and Miguel, 2003) and quinones (Cho et al., 2004) in the DEP samples was determined by previously described procedures. The DTT-based redox activity (Cho et al., 2005) and the DHBA-based Fenton reaction (Di Stefano et al., 2009) assays have also been reported as has the GAPDH-based electrophile assay (Shinyashiki et al., 2008).

2.4. Cellular assay

2.4.1. HO-1 induction assay

The induction of the stress protein HO-1, which has been shown to be a sensitive marker for assessing particulate matter-induced cellular oxidative stress, was found to correlate with DTT activity in a study of ambient air samples collected in the Los Angeles Basin (Li et al., 2003b). This assay was applied to the DEP4 extract and the product of its reductive acetylation. Briefly, 1.5×10^6 murine macrophages (RAW 264.7) were treated with 10, 25 and 50 μg/ml of DEP extracts for 16 h before harvested for immunoblotting of HO-1 protein. The control group received an equal volume of vehicle (DMSO). Electrophoresis of total cellular protein and immunoblotting of HO-1 protein were performed as previously described (Li et al., 2003b).

3. Results and discussion

Our initial analyses were directed at two groups of key organic compounds, the PAHs and representative quinones, 1,2- and 1,4-naphthoquinone (1,2- and 1,4-NQ), 9-10-phenanthraquinone (PQ) and 9,10-anthraquinone (AQ). Quinones are directly formed by combustion of gasoline and diesel fuels (Jakober et al., 2007), and also converted from PAHs either by photochemistry (Eiguren-Fernandez, 2008b) or cellular metabolism (Penning et al., 1999; Zheng et al., 1997). The quinone assay used here estimates the concentration of four representative quinones. DCM extracts of the DEP samples were analyzed for these compounds to relate these samples to other DEPs and ambient air samples. The results are described under Chemical composition. The toxicologically significant property of these and other particles is their ability to

generate reactive oxygen and to form covalent bonds with tissue nucleophiles. Three assays were used to characterize these chemical properties and the results are described under Chemical reactions. The physical properties of the reactive species are relevant to toxicokinetics, since their distribution between particulate and aqueous phases dictates cellular access. The distribution of the assayed reactivities between the particle and aqueous phase of a water suspension was determined and described under Physical properties of reactive species. Finally, the nature of the functional groups in the organic extract involved in the reactions assayed was assessed by reductive acetylation. This procedure converts quinone functions to the less-reactive diacetylhydroquinones. The results of reductive acetylation on the assayed reactivities are described in Chemical properties of organic extracts.

3.1. Chemical composition

3.1.1. PAHs and quinones

The lower-molecular-weight PAHs, phenanthrene (PHE), fluoranthene (FLT) and pyrene (PYR) showed the highest concentrations accounting for more than 90% of the total PAHs (Table 1) and when present, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene, were in very low concentrations. Of the samples analyzed here, DEP4 had the highest PAH content of 790 μg/g, which was 5.6 times higher than the lowest content found in DEP7, which was 137 μg/g. The volatile PAH, naphthalene, was not detected, presumably because it is lost in the collection process, which was limited to particles.

Concentrations of four quinones in the samples are shown in Table 1. As expected, concentrations of somewhat volatile 1,2- and 1,4-NQ were significantly lower than the three-ring quinones, PQ and AQ. Previous studies conducted in the Los Angeles Basin found that 1,2- and 1,4-NQ were mostly in the vapor phase, whereas the three-ring quinones were associated with $PM_{2.5}$ (Eiguren-Fernandez et al., 2008a). DEP4, again, showed the highest quinone content among the seven samples. The concentrations of the same quinones found in particles from other sources are presented for comparison. With the exception of DEP4, the levels of naphthoquinones found in the EPA samples were lower than those reported earlier (Cho et al., 2004; Valavanidis et al., 2006), which may have resulted from using different engines and different fuel composition.

Correlation between PAH and quinone concentrations was high ($r^2 = 0.86$), indicating that quinones are also emitted as primary products of combustion processes, apart from being formed by photochemistry.

3.2. Chemical reactions

Three assays for chemical reactivity were performed. One accesses redox activity based on the ability of the sample to transfer electrons from DTT to oxygen. In this reaction, components of the test sample act as catalysts, accepting electrons from DTT and transferring them to oxygen (Cho et al., 2005; Kumagai et al., 2002). In studies of ambient particles collected in the Los Angeles Basin, a correlation between this activity and PAH content has been observed (Li et al., 2003b) and in particles collected in Fresno, CA, a correlation with quinones was reported (Chung et al., 2006). A second redox assay determines the capacity of the sample to catalyze the Fenton reaction, based on the formation of DHBA isomers from salicylic acid reaction with hydroxyl radical (Coudray and Favier, 2000; Themann et al., 2001). This reaction is limited to transition metal ions such as copper, iron and vanadium, with copper as the most active species (Di Stefano et al., 2009). The third assay reflects the content of electrophiles and is based on the ability of the sample to inhibit or inactivate the thiolate enzyme, GAPDH (Shinyashiki et al., 2008). This assay utilizes the reaction between electrophilic substances present in the sample with the thiolate function in the catalytic center of GAPDH to form covalent bonds, thereby inactivating the enzyme. The commonly used electrophile, NEM, serves as a positive control to monitor the reaction in each assay. The results from this assay are expressed in terms of the activity of NEM, i.e., the number of NEM equivalents per μg sample.

3.3. Physical properties of reactive species

The physical chemical nature of the reactive components of the DEPs was examined by following the three reactivities in an aqueous suspension and its filtrate. Approximately 90% of the DTT-based redox activity was associated with the particles (Table 2). The DHBA activity was found to be exclusively associated with the particles; no activity was found in the filtrates. In contrast, the majority of the GAPDH inhibition-based electrophiles were found in the filtrates, suggesting that highly polar, water-soluble materials in the DEP are responsible for this effect.

At high concentrations, metal ions can oxidize DTT (Kachur et al., 1997; Netto and Stadtman, 1996). Accordingly, the role of metal ions in the DTT-based redox activity was determined by

Table 1
PAH and quinone concentrations (μg/g) in diesel exhaust particle extracts (DEP).

	PHE	FLT	PYR	BAA	BAP	BGP	IND	1,2-NQ	1,4-NQ	PQ	AQ
DEP 2	66.2	56.3	35.4	16.1	0.99	<LOD[a]	<LOD	1.06	0.91	6.02	27.20
DEP 3	115	95.8	60.7	15.8	0.53	<LOD	<LOD	1.77	1.26	8.05	41.90
DEP 4	376	218	180	<LOD	1.44	8.14	6.51	26.04	23.00	14.00	80.30
DEP 5	87.6	179	74.3	12.8	0.80	3.02	4.75	5.15	2.07	9.27	66.00
DEP 6	66.9	141	47.7	6.07	1.08	<LOD	<LOD	4.98	2.46	18.29	66.60
DEP 7	45.3	58.8	29.5	2.92	0.18	0.60	<LOD	4.11	1.88	8.84	46.30
DEP mix	132	138	60.5	<LOD	0.55	<LOD	<LOD	3.86	1.67	13.80	59.20
Japan[b]	1576	678	530	91.0	16.0	18.0	<LOD	22.30	19.90	18.70	69.30
Europe[c]	n/a[d]	n/a	n/a	n/a	n/a	n/a	<LOD	52.70	20.30	3.50	57.90

Abbreviations: PHE, phenathrene; FLT, fluoranthene; PYR, pyrine; BAA, benz[a]anthracene; BAP, benzo[a]pyrene; BGP, benzo[ghi]pyrene; IND, indeno[1,2,4-cd]pyrene; 1,2-NQ, 1,2-naphthoquinone; 1,4-NQ, 1,4-naphthoquinone; PQ, 9,10-phenanthraquinone; AQ, 9,10-anthraquinone.
Concentrations of the indicated quinones in the current study and in DEP from other sources.

[a] <LOD: below limit of detection.
[b] Concentrations found by Cho et al. (2004).
[c] Concentrations found by Valavanidis et al. (2006).
[d] n/a: not available.

comparing DTT activity in the presence and absence of DTPA, a metal chelator that binds copper and iron ions and prevents redox activity. As the Fenton reaction is catalyzed by transition metals, DHBA formation should be completely blocked by DTPA. The results, shown in Table 3, indicated that about 45% of the DTT-based redox activity of the suspension was metal dependent. In contrast, DHBA-based activity is completely DTPA sensitive.

To assess the polarity of these activities, the DCM extracts of the particles, reconstituted in DMSO to allow water solubility, were assayed and the results compared with the aqueous suspension (Table 4). The DCM extracts did not exhibit DHBA-based redox activity, but both DTT redox and GAPDH inhibition activity were observed. The aqueous suspension exhibited approximately 3 times greater DTT activity than the DCM extract. The GAPDH inhibitory activity was also greater in the aqueous suspension, indicating that a significant fraction of the electrophiles was water soluble.

These results show that the redox properties of the DEP samples are associated with the particle fraction when the particles are suspended in water, but that organic substances capable of exhibiting redox activity are also extractable with DCM. These organic substances appear to partition extensively with the particles in an aqueous suspension. In contrast, the electrophiles present in the DEP include both DCM extractable and water soluble species.

3.4. Chemical properties of organic extracts

3.4.1. Reductive acetylation

To assess the role of quinone-like compounds in the reactivities measured, the samples were treated with zinc and acetic anhydride under the conditions used in our quinone assay (Cho et al., 2004). This procedure converts the quinones to their corresponding hydroquinone diacetates. As the diacetates, the hydroquinones are not as susceptible to oxidation, quenching the ability to generate ROS and the α,β-unsaturated carbonyl structure associated with an electrophilic center is absent. This reductive acetylation procedure was applied to the DCM extracts and the resulting changes in DTT-based redox activity and electrophile content determined (Table 5). Although DTT activity was lost completely, some residual electrophilic activity remained, which varied with the sample. The loss of electrophile content varied from 90% to 75%, with the greatest loss by DEP4 and the least by DEP2. These two samples had the highest and the lowest content of organic species, respectively, as evidenced by PAH and quinone content (Table 1).

Table 2
Activities of suspension and filtrates.

Assay	Suspension	Filtrate	Particle	% in particles
DTT activity	21.1 ± 10.9	2.15 ± 2.18	18.9 ± 9.86	90
DHBA activity	1.08 ± 0.54	0.00	1.08 ± 0.54	100
GAPDH activity	29.1 ± 11.3	22.1 ± 7.59	6.94 ± 5.84	24

The activities of the seven samples in Table 1 were determined by the procedures described in Materials and methods. The particle activities were determined from the differences between the suspension and filtrate. The average and standard deviations for the individual assays are shown. The units for DTT activity are pmoles DTT consumed/min/μg sample, those for DHBA are pmoles DHBA formed/min/μg sample and those for GAPDH are % of 1 ng NEM inhibition/μg sample.

Table 3
Role of metals in redox activity of suspensions.

Assay	Aqueous suspension	Suspension+DTPA	% DTPA sensitive
DTT activity	21.1 ± 10.9	11.5 ± 6.29	45
DHBA activity	1.08 ± 0.54	0.00	100

The activities of the seven samples in Table 1 were determined by the procedures described in Materials and methods. The average and standard deviations for the individual assays are shown. The units for DTT activity are pmoles DTT consumed/min/μg sample and those for DHBA are pmoles of DHBA formed/min/μg sample.

3.4.2. Effect of reductive acetylation on HO-1 induction capacity of DEP4

The effect of reductive acetylation on biological activity was examined with DEP4. This sample had the highest content of PAHs and quinones (Table 1) and exhibited high redox as well as electrophilic activity. A DCM extract of this sample was subjected to the reductive acetylation procedure and the ability of the chemically modified extract to induce the stress protein, HO-1, was compared with that prior to chemical modification. The results are shown in Fig. 1, and demonstrate that reductive acetylation reduced the ability of the sample to induce HO-1. Since both redox and electrophilic activity were decreased in the chemically modified sample, it is not possible to distinguish between either of the chemical reactivities as the cause of HO-1 induction. Nevertheless, as quinones are inactivated by the procedure, the results with the DCM extracts indicate that quinones or compounds with similar properties are responsible for HO-1 induction in these preparations.

4. Conclusions

Airborne pollutants are a complex mixture of a large number of chemical entities, and identification of the specific chemicals responsible for the induction of oxidative stress is extremely difficult. However, quantitative measures of toxicologically relevant chemical reaction capacities of pollutants are needed for studies comparing pollutants from different sources and under different atmospheric conditions. With these issues in mind, assays for relevant reaction capacities have been developed and applied to ambient air samples and particles from different sources (Cho et al., 2005; Li et al., 2004; Ntziachristos et al., 2007; Shinyashiki et al., 2008). This study utilized a set of seven DEPs of varying chemical content to assess the physical properties of those constituents that carry out these reactions by determining their distribution between the particle and water phase in a suspension and the DCM solubility of the reactive constituents. In addition, the DCM extract, which contains the organic constituents, was subjected to a reductive acetylation procedure to

Table 4
Comparison of aqueous suspension (aqueous) and DCM (organic) extract.

	DTT activity (pmol/min/μg)		DHBA formation (pmol/min/μg)		GAPDH (% of 1 ng NEM/μg)	
	Organic (DCM) extract	Aqueous suspension	Organic (DCM) extract	Aqueous suspension	Organic (DCM) extract	Aqueous suspension
Average ± SD of seven samples	5.95 ± 2.12	21.1 ± 10.9	Not measured	1.08 ± 0.54	19.5 ± 10.8	29.1 ± 11.3

Table 5
Effects of reductive acetylation of organic extracts.

	DTT activity (pmol/min/μg)		GAPDH inhibitory activity (% of 1 ng NEM/μg of original mass)		% GAPDH inhibition affected by acetylation
	Control	Zn+Ac_2O	Control	Zn+Ac_2O	
Average ± SD of seven samples	5.95 ± 2.12	0.00	19.5 ± 10.8	2.74 ± 0.78	86

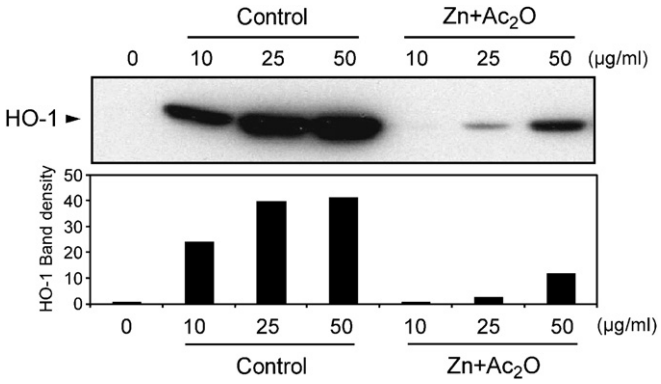

Fig. 1. Heme oxygenase-1 (HO-1) induction by the dichloromethane (DCM) extract of DEP 4. Raw 264.7 cells were incubated with the extract without (control) or with reductive acetylation (Zn+Ac_2O) for 16 h at the indicated concentrations.

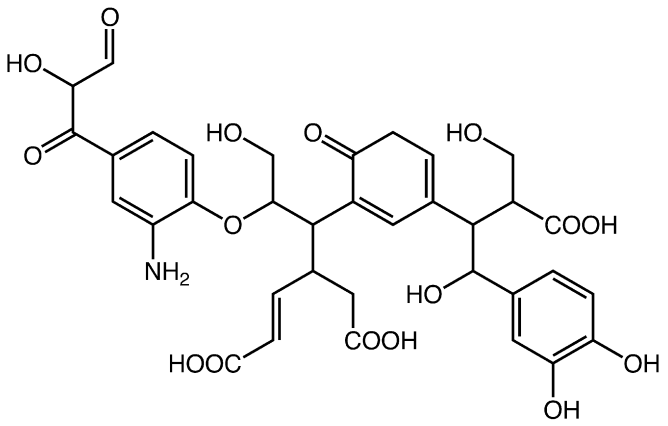

Fig. 2. Humic acid building block (from Steelink, 1963).

evaluate the role of quinone-like compounds in the observed activities.

Exposure to ambient air particles and vapors results in a suspension of the mixture in the lung-lining fluid. The soluble materials will dissolve and can enter cells either by transport or by passive diffusion. Particles can enter the cell by phagocytosis and other (Geiser et al., 2005; Moller et al., 2008) processes and exert their chemical effects either through the particle or through the dissolution of adsorbants within the cell. Particles can also generate reactive oxygen extracellularly and cause cellular damage. These toxicokinetic issues require an understanding of the distribution of the reactive constituents between the particle and aqueous phase. Fractionation of the aqueous suspension into filtrate and filter-bound particles showed that redox activity was essentially bound to the particle phase, whereas the electrophiles were distributed between both phases. Since particles will distribute differently from solutes, the distribution of redox active species will move with the particles, whereas the electrophiles will be likely more diffuse in their distribution.

The partition of metal-based redox active species in the particle phase suggests that metals are bound to the particles, possibly by chelation with some structure on the particles. This process could take place with humic acid like functions on the particles. DEP are known to contain humic-like substances (HLS) (Ghio et al., 1996), compounds or structures related to humic acids, which are polymers of multifunctional aromatic and aliphatic monomers (Steelink 1963) and can contain catechol structures which would exhibit redox activity, α,β-unsaturated carbonyl systems which would exhibit electrophilic activity and multiple carboxylate functions which could act as metal chelators (see Fig. 2). These structures are thought to be formed in the course of fuel combustion (Ghio et al., 1996).

If some of the HLS were extractable from the particles with DCM the redox and electrophile content may be explainable. These functionalities would also be inactivated by the reductive acetylation procedure. The redox and electrophilic properties of the DCM extract, which contains only organic compounds, were lost or substantially reduced after reductive acetylation, suggesting that the observed activities must be due to quinones or quinone-like chemical species. In contrast to redox activity, the reduction in electrophile content following reductive acetylation varied with the samples, ranging from 75% to 90%. This variability may reflect the differences in the content and nature of electrophiles in the samples. The chemical analyses of the samples also showed differences; for example, the naphthoquinone content of DEP4 was almost 10-fold greater than the next highest and as results of the reductive acetylation experiment indicate that DEP4 had the lowest residual activity, it is likely that quinones such as these are the major contributor to the electrophilicity of this sample. The cellular actions of the DCM extract of DEP4 suggest that the organic species responsible for HO-1 induction also exhibit the same chemical properties.

Electrophiles are a particularly important component of air pollution because of their covalent bond forming ability. They will form covalent bonds with a variety of nucleophiles such as thiolates, which is the key functional group in glutathione as well as the many enzymes who utilize the nucleophilic properties of thiolate to catalyze reactions. Once formed, the affected chemical species is irreversibly inactivated and must be replaced in the cell by renewed synthesis. The recovery by the cell from a covalent bond-based insult will therefore depend on protein turnover. Thus, exposure to even a small quantity of electrophiles can result in significant biological effects because the actions on slowly turning over proteins will be cumulative. In related studies, we have demonstrated that 1,2-NQ itself can form covalent bonds with protein tyrosine phosphatases to activate the epidermal growth factor receptor kinase (Iwamoto et al., 2007). This kinase has been associated with the exacerbation of asthma through its involvement in lung remodeling and changes in lung cell morphology that reflect asthma status (Puddicombe et al., 2000).

In summary, these DEPs contain both redox metals and redox active organic substances. The metals appear to be tightly bound to particles, for they are not extractable into water. The particles also contain electrophiles which exhibit both water and

dichloromethane solubility. A likely array of organic functional groups that would account for these properties are those found in humic-like substances. The differences in solubility of the redox active species and the electrophiles will result in different types of exposure. The redox activity would likely enter the cell as a particle-bound electron transfer agent, whereas the soluble electrophiles would enter the cell as individual molecular species.

References

Cho, A.K., Di Stefano, E., You, Y., Rodriguez, C.E., Schmitz, D.A., Kumagai, Y., et al., 2004. Determination of four quinones in diesel exhaust particles, SRM 1649a and atmospheric $PM_{2.5}$. Aerosol Sci. Technol. 38 (S1), 68–81.

Cho, A.K., Sioutas, C., Miguel, A.H., Kumagai, Y., Schmitz, D.A., Singh, M, et al., 2005. Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. Environ. Res. 99, 40–47.

Chung, M.Y., Lazaro, R.A., Lim, D., Jackson, J., Lyon, J., Rendulic, D., et al., 2006. Aerosol-borne quinones and reactive oxygen species generation by particulate matter extracts. Environ. Sci. Technol. 40, 4880–4886.

Coudray, C., Favier, A., 2000. Determination of salicylate hydroxylation products as an in vivo oxidative stress marker. Free Radical Biol. Med. 29, 1064–1070.

Dagher, Z., Garcon, G., Billet, S., Gosset, P., Ledoux, F., Courcot, D., et al., 2006. Activation of different pathways of apoptosis by air pollution particulate matter ($PM_{2.5}$) in human epithelial lung cells (L132) in culture. Toxicology 225, 12–24.

Di Stefano, E., Eiguren-Fernandez, A., Delfino, R.J., Sioutas, C., Froines, J.R., Cho, A.K., 2009. Determination of metal-based hydroxyl radical generating capacity of ambient and diesel exhaust particles. Inhal. Toxicol., in press.

Donaldson, K., Stone, V., Borm, P.J., Jimenez, L.A., Gilmour, P.S., Schins, R.P., et al., 2003. Oxidative stress and calcium signaling in the adverse effects of environmental particles (PM_{10}). Free Radical Biol. Med. 34, 1369–1382.

Eiguren-Fernandez, A., Miguel, A., 2003. Determination of semivolatile and particulate polycyclic aromatic hydrocarbons in SRM 1649a and $PM_{2.5}$ samples by HPLC-fluorescence. Polycycl. Aromat. Compd. 23, 193–205.

Eiguren-Fernandez, A., Miguel, A., Di Stefano, E., Schmitz, D., Cho, A., Thurairatnam, S., et al., 2008a. Atmospheric distribution of gas- and particle-phase quinones in Southern California. Aerosol Sci. Technol. 42, 854–861.

Eiguren-Fernandez, A., Miguel, A.H., Lu, R., Purvis, K., Grant, B., Mayo, P., et al., 2008b. Atmospheric formation of 9,10-phenanthraquinone in the Los Angeles Air Basin. Atmos. Environ. 42, 2312–2319.

Geiser, M., Rothen-Rutishauser, B., Kapp, N., Schurch, S., Kreyling, W., Schulz, H., et al., 2005. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ. Health Perspect. 113, 1555–1560.

Ghio, A.J., Stonehuerner, J., Pritchard, R.J., Piantadosi, C.A., Quigley, D.R., Dreher, K.L., et al., 1996. Humic-like substances in air pollution particulates correlate with concentrations of transition metals and oxidant generation. Inhal. Toxicol. 8, 479–494.

Imrich, A., Ning, Y., Lawrence, J., Coull, B., Gitin, E., Knutson, M., et al., 2007. Alveolar macrophage cytokine response to air pollution particles: oxidant mechanisms. Toxicol. Appl. Pharmacol. 218, 256–264.

Iwamoto, N., Sumi, D., Ishii, T., Uchida, K., Cho, A.K., Froines, J.R., et al., 2007. Chemical knockdown of protein tyrosine phosphatase 1B by 1,2-naphthoquinone through covalent modification causes persistent transactivation of epidermal growth factor receptor. J. Biol. Chem. 282, 33396–33404.

Jakober, C.A., Riddle, S.G., Robert, M.A., Destaillats, H., Charles, M.J., Green, P.G., et al., 2007. Quinone emissions from gasoline and diesel motor vehicles. Environ. Sci. Technol. 41, 4548–4554.

Kachur, A.V., Held, K.D., Koch, C.J., Biaglow, J.E., 1997. Mechanism of production of hydroxyl radicals in the copper-catalyzed oxidation of dithiothreitol. Radiat. Res. 147, 409–415.

Kumagai, Y., Koide, S., Taguchi, K., Endo, A., Nakai, Y., Yoshikawa, T., et al., 2002. Oxidation of proximal protein sulfhydryls by phenanthraquinone, a component of diesel exhaust particles. Chem. Res. Toxicol. 15, 483–489.

Li, N., Alam, J., Venkatesan, M.I., Eiguren-Fernandez, A., Schmitz, D., Di Stefano, E., et al., 2004. Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals. J. Immunol. 173, 3467–3481.

Li, N., Hao, M., Phalen, R.F., Hinds, W.C., Nel, A.E., 2003a. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. Clin. Immunol. 109, 250–265.

Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., et al., 2003b. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ. Health Perspect. 111, 455–460.

Moller, W., Felten, K., Sommerer, K., Scheuch, G., Meyer, G., Meyer, P., et al., 2008. Deposition, retention, and translocation of ultrafine particles from the central airways and lung periphery. Am. J. Respir. Crit. Care Med. 177, 426–432.

Netto, L.E., Stadtman, E.R., 1996. The iron-catalyzed oxidation of dithiothreitol is a biphasic process: hydrogen peroxide is involved in the initiation of a free radical chain of reactions. Arch. Biochem. Biophys. 333, 233–242.

Ntziachristos, L., Froines, J.R., Cho, A.K., Sioutas, C., 2007. Relationship between redox activity and chemical speciation of size-fractionated particulate matter. Part. Fibre Toxicol. 4, 5.

Penning, T.M., Burczynski, M.E., Hung, C.F., McCoull, K.D., Palackal, N.T., Tsuruda, L.S., 1999. Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones. Chem. Res. Toxicol. 12, 1–18.

Puddicombe, S.M., Polosa, R., Richter, A., Krishna, M.T., Howarth, P.H., Holgate, S.T., et al., 2000. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. FASEB J. 14, 1362–1374.

Rahman, I., Biswas, S.K., Kode, A., 2006. Oxidant and antioxidant balance in the airways and airway diseases. Eur. J. Pharmacol. 533, 222–239.

Rodriguez, C.E., Fukuto, J.M., Taguchi, K., Froines, J., Cho, A.K., 2005. The interactions of 9,10-phenanthrenequinone with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a potential site for toxic actions. Chem. Biol. Interact. 155, 97–110.

Shinyashiki, M., Rodriguez, C.E., Di Stefano, E.W., Sioutas, C., Delfino, R.J., Kumagai, Y., et al., 2008. On the interaction between glyceraldehyde-3-phosphate dehydrogenase and airborne particles: evidence for electrophilic species. Atmos. Environ. 42, 517–529.

Steelink, C., 1963. What is humic acid? J. Chem. Educ. 40, 379–384.

Themann, C., Teismann, P., Kuschinsky, K., Ferger, B., 2001. Comparison of two independent aromatic hydroxylation assays in combination with intracerebral microdialysis to determine hydroxyl free radicals. J. Neurosci. Methods 108, 57–64.

Valavanidis, A., Fiotakis, K., Vlahogianni, T., Papadimitriou, V., Pantikaki, V., 2006. Determination of selective quinones and quinoid radicals in airborne particulate matter and vehicular exhaust particles. Environ. Chem. 3, 118–123.

Zheng, J., Cho, M., Jones, A.D., Hammock, B.D., 1997. Evidence of quinone metabolites of naphthalene covalently bound to sulfur nucleophiles of proteins of murine Clara cells after exposure to naphthalene. Chem. Res. Toxicol. 10, 1008–1014.